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ARTICLE

Degradation of Sulfonamides in Aqueous Solution by Membrane Anodic Fenton Treatment

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Two agricultural antibiotics used heavily in agriculture, sulfamethazine and sulfadiazine, were degraded in an aqueous system by anodic Fenton treatment (AFT), an advanced oxidation technique that has been shown to be effective in degrading various pesticides but has not been applied to antibiotics. The effects of the H_2O_2/Fe^{2+} ratio, Fe^{2+} delivery rate, and initial contaminant concentration on the degradation of sulfamethazine by AFT were determined. The optimal H_2O_2/Fe^{2+} ratio was determined to be 10:1, and the optimal Fe^{2+} delivery rate was found to be between 38.9 and 54.4 μM min^{-1}. Under these conditions, sulfamethazine was completely degraded within 10 min at a range of concentrations (18−250 μM) commonly found in manure lagoons, contaminated rivers, and groundwater. Using the same optimal conditions, the effect of pH on the degradation of sulfadiazine by AFT was analyzed, and 100 μM sulfadiazine was degraded within 6−8 min of treatment at a range of pH values (3.1−7.1) that could potentially be found in aquatic environments. Degradation products and pathways were proposed for both compounds, and it was inferred that AFT degradation products of sulfadiazine and sulfamethazine are unlikely to retain the bacteriostatic properties of their parent compounds. An aquatic toxicity test employing *Lemna gibba* confirmed that AFT removes the bacteriostatic properties of sulfamethazine and sulfadiazine during degradation.

KEYWORDS: Sulfamethazine; sulfadiazine; anodic Fenton treatment; hydrogen peroxide; oxidation; wastewater; antibiotics; agricultural waste; *Lemna gibba*

INTRODUCTION

Seventy percent of the 23000 tons of antibiotics that are used each year are used as agricultural growth promoters, because they improve nutrient uptake in the gastrointestinal tract (*1*). Large quantities of unmetabolized antibiotics enter the soil as a result of the application of agricultural manure to fields to improve soil fertility (*1*, *2*). Antibiotics and their metabolites that do not strongly adhere to soil particles may leach into groundwater or enter surface water via runoff. Their presence in the soil and aquatic environment at subtherapeutic concentrations may promote the growth of antibiotic-resistant bacteria and may alter ecosystem structure by reducing the numbers of soil bacteria (*1*,*3*).

Sulfonamides are a class of bacteriostatic antibiotics frequently used in agriculture. They weakly adsorb to soil particles, have the potential to leach into groundwater, and have been detected in agricultural manure lagoons and in the aquatic environment (*1*, *2*, *4*). Sulfamethazine has been detected in European and U.S. manure lagoons at concentrations of approximately 143.7 and 1.4 μM, respectively (*1*, *5*). Concentrations of sulfamethazine below the therapeutic range of approximately 1.8−7.2 μM pose the greatest risk for supporting the growth of resistant organisms in the environment (*1*). Because the average concentration of sulfamethazine in U.S. rivers ($\sim 1.8 \times 10^{-4}$ μM) is below this range, sulfamethazine may promote resistance in the aquatic environment (*1*). Sulfadiazine is also used heavily in agriculture and has been detected in agricultural waste and in the environment (*6*, *7*); its similarity in chemical structure to sulfamethazine makes it an ideal candidate for comparative degradation analysis.

Sulfonamides are structurally similar to *p*-aminobenzoic acid (PABA), a compound used by bacteria to synthesize folic acid, an important component in DNA synthesis (**Figure 1**). By competing with PABA for binding sites on dihydropteroate synthase (DHPS), an enzyme necessary in folic acid synthesis, they inhibit the growth of bacteria (*8*). Sulfonamides and PABA both have a substituent on the primary carbon, a sulfonyl group (SO_2) in the case of sulfonamides and a carbonyl group (COOH) in the case of PABA, as well as a primary amine group (NH_2) on the aromatic ring. The aromatic primary amine group is very active in the first step in folic acid synthesis, and its activity, and thus the bacteriostatic activity of the sulfonamide, is affected by the properties of the substituent on the primary carbon (*9*).

Advanced oxidation treatments that produce hydroxyl radicals, such as those involving ozonation, hydrogen peroxide plus UV light, and ferric ion plus UV light, have been well studied (*10*−*13*). One oxidation treatment that shows promise for on-site degradation of contaminants in water uses a combination of ferrous ion and hydrogen peroxide, known as Fenton's reagent. Systems employing Fenton's reagent are simple to operate and require reasonably priced equipment (*10*). The kinetics of degradation

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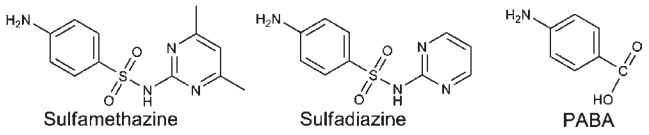

Figure 1. Structures of sulfamethazine and PABA. Note the similar C^1 substituents and aromatic NH_2 groups on both compounds.

of several pesticides by Fenton's reagent has been well researched (*10*, *14*−*16*), and Fenton's reagent has been incorporated into several treatment scenarios, such as photo-Fenton treatment (*17*), electrochemical Fenton treatment (*18*), cathodic Fenton treatment (*19*), and anodic Fenton treatment (*20*). Anodic Fenton treatment (AFT) is one of the most promising of these Fenton treatments, as it is highly efficient, has a fairly neutral effluent, and can easily be applied to contaminated water (*20*).

This research extends the application of AFT, which has been shown to be effective in degrading various pesticides (*21*, *22*), to sulfonamide antibiotics in an aqueous solution. The overall goal is to develop treatments that will degrade antibiotics in agricultural waste before their application to fields and to remediate water that is already contaminated.

The specific objectives of this research are (i) to determine the kinetics of AFT-mediated degradation of sulfamethazine and sulfadiazine in an aqueous system, (ii) to optimize the AFT system for these two compounds, (iii) to propose degradation pathways for the tested sulfonamide antibiotics on the basis of their degradation products, and (iv) to use the aquatic plant *Lemna gibba* as a model for testing the bacteriostatic properties of the degradation products in the AFT effluent.

MATERIALS AND METHODS

Chemicals. Sulfamethazine (4-amino-*N*-(4,6-dimethyl-2-pyrimidinyl) benzenesulfonamide) (99%) and sulfadiazine (4-amino-*N*-(2-pyrimidinyl) benzenesulfonamide) (99%) were purchased from Sigma-Aldrich (St. Louis, MO). Hydrogen peroxide (30%, analytical grade) and sodium chloride (crystal) were purchased from Mallinckrodt-Baker, Inc. (Paris, KY). Acetonitrile (HPLC grade), water (HPLC grade), and NaOH (98%) were purchased from Fisher Chemicals (Fair Lawn, NJ). Phosphoric acid (85%, analytical grade) and hydrochloric acid (36.5−38%, analytical grade) were purchased from Mallinckrodt-Baker, Inc. (Phillipsburg, NJ).

Membranes. The anion exchange membrane was purchased from Electrosynthesis. The electrical resistance of the anion exchange membrane in 1 M NaCl solution at 25 °C is 8 ohm cm^{-2}.

Toxicity Test Cultures. Duckweed, *L. gibba* (G-3), was obtained from an axenic test culture purchased from the Canadian Phycological Culture Centre at the University of Waterloo (Waterloo, ON, Canada). Cultures were propagated according to a standard method (*23*) but were grown in autoclaved Hoagland's media, prepared from a Hoagland-modified basal salt mixture that was purchased from PhytoTechnology Laboratories (Shawnee Mission, KS) dissolved in HPLC grade water. Cultures were grown at 23 °C under 24 h fluorescent light (6800 lx), which was measured by a Reed LM-81LX digital light meter purchased from Calright Instruments (San Diego, CA).

Degradation of Sulfonamides by Membrane Anodic Fenton Treatment. The AFT setup consisted of a 600 mL glass anodic half-cell containing 400 mL of 100 μM sulfamethazine or sulfadiazine connected by an ion exchange membrane to a 600 mL cathodic half-cell containing 400 mL of 0.08 M NaCl (*21*). Sulfamethazine was added to the anodic half-cell from a stock solution of 1000 μM sulfamethazine in distilled water, and sulfadiazine originated from a stock solution of 1000 μM sulfadiazine in distilled water acidified with hydrochloric acid (for dissolution purposes). Both solutions were stirred with magnetic stir bars. When electrolysis was applied to the anodic half-cell at a current of 0.05 A, the iron anode (a 2 cm × 10 cm × 0.2 cm iron plate) was oxidized and ferrous ion was delivered to the anodic half-cell at a rate of 38.9 μM min^{-1}. At the same time, in the cathodic half-cell, water was reduced on a 1 cm × 10 cm graphite cathode to hydrogen gas and hydroxide ions (eqs **1** and **2**) (*21*). Electricity was supplied by a BK Precision DC power supply 1610 at 0.05 A. Simultaneously, a hydrogen peroxide solution of 0.311 M, prepared by dilution of a known concentration and determined by titration in an acidic solution using a standard potassium permanganate solution (*23*), was delivered to the anodic half-cell by a Stepdos 08S pump (Chemglass, Inc.) at a rate of 0.5 mL min^{-1} (*21*). The addition of hydrogen peroxide to the anodic half-cell is known as the Fenton reaction (eq **3**) (*21*), which produces hydroxyl radicals that degrade the compound.

$$\text{anode}: \quad Fe \rightarrow Fe^{2+} + 2e^- \tag{1}$$

$$\text{cathode}: \quad 2H_2O + 2e^- \rightarrow H_2 + 2OH^- \tag{2}$$

$$Fe^{2+} + H_2O_2 = Fe^{3+} + {}^{\bullet}OH + OH^- \tag{3}$$

Because the ratio of the delivery rate of hydrogen peroxide to the delivery rate of ferrous ion can affect degradation rate and efficiency (*21*), all initial experiments were performed at a fixed H_2O_2/Fe^{2+} ratio of 10:1, based on optimization studies (vide infra). Experiments were run at the ambient temperature, ∼20 °C. Power was delivered when the first drop of hydrogen peroxide solution reached the anodic half-cell. Samples (1.0 mL) of the solution from the anodic half-cell were collected at specific times over 15 min of treatment and put into glass high-performance liquid chromatography (HPLC) vials containing 0.1 mL of methanol, which quenched the hydroxyl radical and prevented further degradation of the compound (*21*). The treatments were repeated in triplicate, and the samples were analyzed using HPLC and LC-MS.

Optimization of AFT Degradation of Sulfamethazine and Sulfadiazine. To determine the effect of the H_2O_2/Fe^2 ratio on AFT degradation of sulfamethazine, AFT was performed with various H_2O_2/Fe^{2+} ratios between 1:1 and 20:1 (*21*). In addition, the effect of the Fe^{2+} delivery rate on degradation was determined by applying AFT to sulfamethazine with Fe^{2+} delivery rates between 7.89 and 78 μM min^{-1}. To determine the effect of initial concentration on degradation rate, AFT was applied to sulfamethazine solutions with concentrations between 18 and 250 μM at a H_2O_2/Fe^{2+} ratio of 10:1 and a Fe^{2+} delivery rate of 38.9 μM min^{-1}.

Unlike sulfamethazine, sulfadiazine required the addition of acid to dissolve in water, which resulted in a stock solution pH of 2.3. Because AFT typically works best at pH 3.1 (*20*), the effect of initial pH on the degradation rate of sulfadiazine was studied by applying AFT to sulfadiazine solutions with initial pH values between 3.1 and 7.1. Due to the structural similarity of sulfamethazine to sulfadiazine, the optimal conditions for AFT degradation were also used for sulfadiazine. The initial pH of each solution was adjusted by adding 1 M HCl and was measured using an Accumet Basic AB15 pH-meter (Fisher Scientific).

Determination of Sulfamethazine and Sulfadiazine Concentrations. The concentration of sulfamethazine in each sample was quantified using an Agilent HPLC 1100 series with a diode array detector set at 254 nm and an Ultra C18 column (4.6 mm × 250 mm) with a particle size of 5 μm and pore size of 100 Å. The mobile phase consisted of 75:25 water (adjusted to pH 3 with phosphoric acid)/acetonitrile. The injection volume was 20 μL, and the flow rate was 1 mL min^{-1}. Sulfamethazine had a retention time of 5.7 min under these conditions. The concentration of sulfadiazine was quantified using an Agilent HPLC 1200 series with a diode array detector set at 254 nm and the same mobile phase described above. A Zorbax Eclipse XDB-C18 column (4.6 mm × 150 mm) with a particle size of 5 μm was used (rapid resolution liquid chromatography (RRLC)). The injection volume was 25 μL, and the flow was 0.5 mL min^{-1}. Sulfadiazine had a retention time of 4.9 min under these conditions.

Determination of Sulfamethazine and Sulfadiazine Degradation Products and Pathways. Each sulfonamide was treated by membrane AFT according to the established optimal conditions, and each sample was prepared as described above. The sample was analyzed by an Agilent LC 1200 series with 6130 quadrapole LC-MS. The LC-MS method consisted of MM-ES+APCI with a positive polarity scan m/z 70 to m/z 350, with the molecule fragmenter voltage set at 110 V. The internal energies and dipoles of the degradation products were analyzed using ab initio equations in the GAMESS program in the ChemBio3D Ultra program to determine the most likely degradation pathways. In order to calculate the internal energy

and dipole of the most likely molecular orientation of each product, energy and geometry of the product was either minimized or its transition state was optimized using closed shell HF with a basis set of 3-21G before the calculations were performed.

Testing the Toxicity of AFT Effluent to *L. gibba*. To test whether AFT removed the bacteriostatic properties of sulfamethazine and sulfadiazine, toxicity tests using the aquatic plant *L. gibba* (duckweed) were performed. Sulfonamides elicit toxic effects by targeting the folate synthesis pathway in bacteria as well as in plants (*24*), and exposure to sulfonamides decreases *L. gibba* wet weight (*25*). Our aim was not to determine the LC_{50} of the AFT effluent, but rather to use the test as a method for determining the point during AFT at which the effluent is no longer toxic to *L. gibba*. This would indicate whether the bacteriostatic properties of the sulfonamide were removed during AFT.

The procedure for the *L. gibba* tests was based on a previously established protocol for testing the toxicity of various compounds to *L. gibba* (*26*, *27*). Experimental units and controls (33 10-mL culture dishes) were arranged on large trays according to a randomized complete block design with 30 experimental units (10 treatments with 3 replicates) and 3 control replicates.

To establish that toxicity to *L. gibba* was due solely to the presence of the sulfonamide, AFT was run with 444 μM sulfamethazine, with 487 μM sulfadiazine, or without a sulfonamide (control). These concentrations would produce concentrations in the culture dishes equivalent to the published EC_{50} value for sulfamethazine and would allow for a clear demonstration of a decrease in effluent toxicity from the beginning of AFT to the end (*25*). There is no published EC_{50} value for sulfadiazine, so AFT was performed with an initial concentration similar to that of sulfamethazine due to the similarity in structure between the two compounds. Controls contained AFT effluent without a sulfonamide to ensure that the hydrogen peroxide, iron, and methanol present in the AFT effluent throughout the experiment did not affect *L. gibba* growth.

The pH values of the anodic half-cell solutions were adjusted accordingly before AFT so that the pH of the final growth medium would be in the recommended pH range (*27*). Effluent (4 mL) was collected at 0, 2, 4, 6, and 15 min, and 0.4 mL of methanol was added. All experimental units and controls were prepared under a laminar flow hood. Experimental units consisted of 10 mL of Hoagland's medium and 0.1 mL of the AFT effluent/methanol solution. Controls consisted of 10 mL of Hoagland's medium with 0.1% methanol, as 0.2% methanol enhances *L. gibba* growth rates (*28*). Two *L. gibba* plants with three fronds each were placed in each culture dish, and plants were transferred daily to corresponding dishes containing fresh solutions. Tests were performed at 23 °C under 24 h cool, fluorescent light (6800 lx) for 7 days. On day 7, plants from each dish were removed, blotted dry, and weighed. Wet mass was the only end point measured, as it was previously found to be the only significant *L. gibba* end point affected by exposure to sulfonamides (*25*). The total number of fronds in each control was counted to ensure the validity of the experiment (*26*, *27*).

Statistical Analyses. Sigmaplot 9.0 (Systat Software, Inc., Richmond, CA) was used for model fitting. Toxicity test data were analyzed using SPSS. Residual normality and homogeneous variance satisfied the assumptions of a one-way analysis of variance (ANOVA). An ANOVA using Dunnett's test was performed on the wet weights of experimental units and controls to determine significant effects ($p < 0.05$) for each tested sulfonamide.

RESULTS AND DISCUSSION

Degradation of Sulfonamides. AFT completely degraded sulfamethazine in an aqueous solution with an H_2O_2/Fe^{2+} ratio of 10:1 within 6 min (**Figure 2**). Controls were run by applying either electricity alone as a source of Fe^{2+} (figure not shown) or hydrogen peroxide alone to anodic half-cell solutions of 100 μM of the sulfonamide. There was no significant degradation under either of these conditions, confirming that oxidation is due solely to AFT.

Kinetic Model of Degradation. It was initially hypothesized that the degradation of sulfamethazine could be explained by the established AFT kinetic model for the degradation of organic compounds (eq **4**), which fits the degradation trend of many pesticides (*20*).

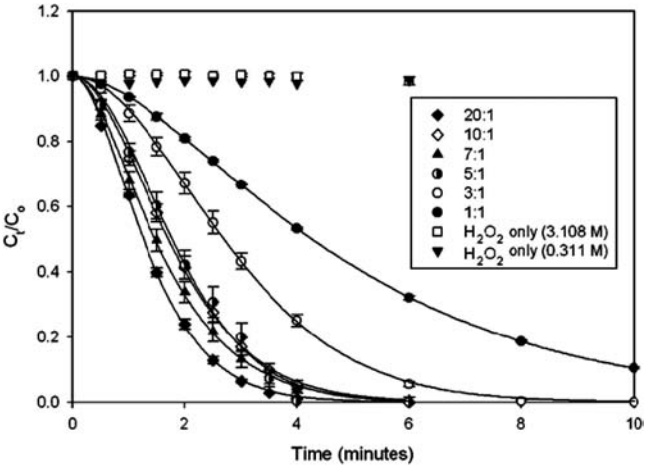

Figure 2. Degradation of 100 μM sulfamethazine by AFT with a Fe^{2+} delivery rate of 38.9 μM min^{-1} and at various H_2O_2/Fe^{2+} ratios, by 0.311 M H_2O_2 alone and by 3.108 M H_2O_2 alone. Data were fit by the modified AFT model (eq **5**).

$$\ln\left(\frac{[C]_t}{[C]_0}\right) = -\frac{1}{2}K\lambda\pi\omega{v_0}^2t^2 \quad (4)$$

In this model, K represents a combination of the second-order rate constants of the Fenton reaction (eq **2**) and the degradation of the compound by hydroxyl radicals (*20*). $[C]_t$ represents the concentration of the compound at time t, $[C]_0$ is initial concentration, and λ is the lifetime of the hydroxyl radical (min) (*20*). π is the lifetime of Fe^{2+} (min), v_0 is the delivery rate of Fe^{2+} by electrolysis (μM min^{-1}), and ω is a constant determined from the ratio of the delivery rate of H_2O_2 to the delivery rate of Fe^{2+} and from the degradation rate of H_2O_2 (*20*). The model assumes that the concentration of Fe^{2+} in the anodic half-cell is constant, hydrogen peroxide accumulates in the anodic half-cell, both the Fenton reaction and the reaction between the hydroxyl radical and the organic compound follow second-order kinetics, and the concentration of hydroxyl radicals is proportional to the rate at which they are generated (*20*).

The AFT model described above did not fit the degradation kinetics of sulfamethazine well, so a second previously established model called the modified AFT model (eq **5**) that describes the degradation of triazines was applied (*22*).

$$\ln\left(\frac{[C]_t}{[C]_0}\right) = -\frac{a}{b^2}\ln\left(\frac{a}{a+bt}\right) - \frac{t}{b} \quad (5)$$

where

$$a = \frac{1}{K\lambda\pi\omega{v_0}^2} \text{ and } b = \frac{\eta\kappa_{D-Fe^{3+}}}{K\lambda\pi\omega v_0}$$

This model shares many of the same parameters as the AFT model, but there are two important differences. η is a constant less than 1 and represents the ratio of the concentration of ferric iron to total iron during the AFT (*22*). $\kappa_{D-Fe^{3+}}$ is the equilibrium constant of $\frac{[D-Fe^{3+}]}{[D]_{free}[Fe^{3+}]}$ where $[D-Fe^{3+}]$ is the concentration of the compound bound to ferric ion and $[D]_{free}$ is the concentration of the unbound compound (*22*). With these parameters, the model accounts for the ability of compounds with unsaturated

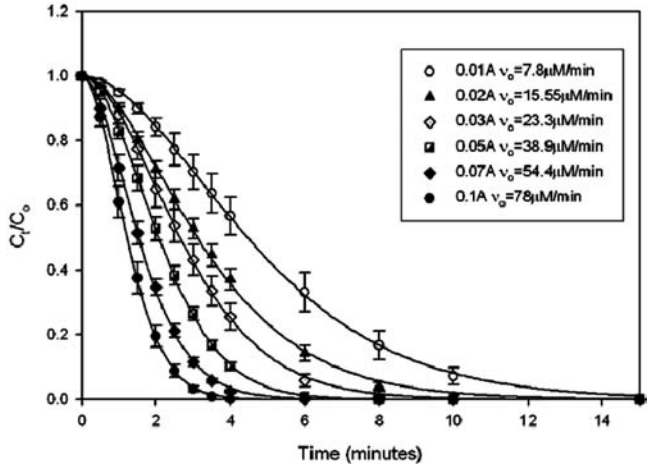

Figure 3. Degradation of 100 μM sulfamethazine by AFT at various Fe^{2+} delivery rates at the optimum H_2O_2/Fe^{2+} of 10:1. Data were fit by the modified AFT model (eq **5**).

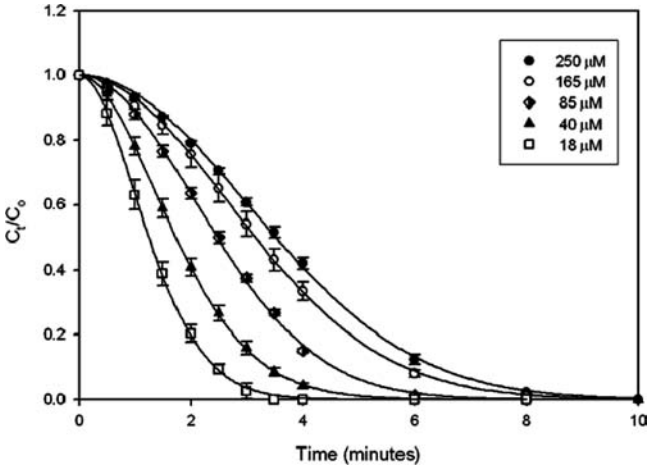

Figure 4. Degradation of sulfamethazine by AFT at various initial concentrations at the optimum H_2O_2/Fe^{2+} of 10:1 and with a Fe^{2+} delivery rate of 38.85 μM min^{-1}. Data were fit by the modified AFT model (eq **5**).

heterocyclic rings containing nitrogen, such as sulfamethazine and sulfadiazine, to form a complex with the ferric ion generated in eq **2**, making the compound less easily degraded by the hydroxyl radical (*22*). As ferric ion accumulates in the system and complexes with the compound, the amount of the compound available for degradation, and thus the amount of the compound that is degraded, decreases (*22*). This model fits the degradation of sulfamethazine well, with a regression coefficient >0.999 (**Figure 2**).

Effect of H_2O_2/Fe^{2+} Ratio and Fe^{2+} Delivery Rate on Degradation of Sulfamethazine. The effects of the initial concentration of the compound, the Fe^{2+} delivery rate, and the H_2O_2/Fe^{2+} ratio on the time to complete degradation were assessed (*21*). The degradation of sulfamethazine at various H_2O_2/Fe^{2+} ratios fit the modified AFT model well, with regression coefficients of >0.999 (**Figure 2**). The degradation of sulfamethazine at various Fe^{2+} delivery rates also fit the revised AFT model well, with regression coefficients of >0.999 (**Figure 3**). Consistent with the model, the time at which the compound was completely degraded decreased as the Fe^{2+} delivery rate increased (**Figure 3**).

When AFT is optimized as a potential water remediation process, the cost-effectiveness of reagents and the environmental consequences of high concentrations of reagents should be considered in addition to the time to complete degradation of the compound. With these factors in mind, the optimum H_2O_2/Fe^{2+} ratio for the degradation of sulfamethazine was inferred to be the lowest ratio at which 100% of the initial concentration of the compound was degraded in the least amount of time. The H_2O_2/Fe^{2+} ratio that fulfilled this requirement was 10:1, at which 100 μM sulfamethazine was degraded in 6 min (**Figure 2**). This ratio is consistent with the optimum H_2O_2/Fe^{2+} ratio determined for other compounds (*21*). The two Fe^{2+} delivery rate conditions that resulted in the lowest times to complete degradation of sulfamethazine were 54.4 μM (6 min) and 38.9 μM (8 min) and required hydrogen peroxide concentrations of 0.435 and 0.311 M, respectively (**Figure 3**). Therefore, an optimal Fe^{2+} delivery rate for the degradation of sulfamethazine would be in the range of 38.9−54.4 μM min^{-1}, because it would require a moderate voltage and hydrogen peroxide concentration and would degrade the compound in a reasonable amount of time.

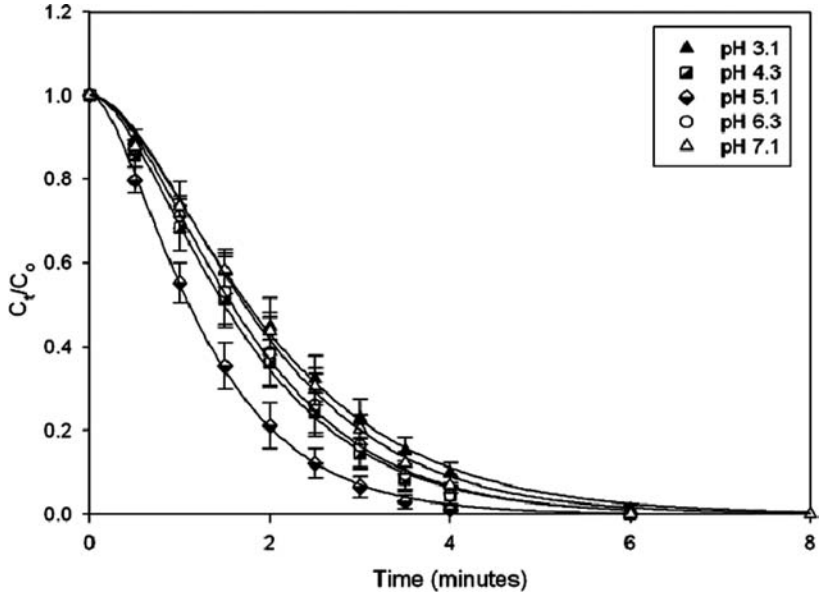

Figure 5. Degradation of 100 μM sulfadiazine at various pH values at an optimum H_2O_2/Fe^{2+} of 10:1 and with a Fe^{2+} delivery rate of 38.9 μM min^{-1}. Data were fit by the modified AFT model (eq **5**).

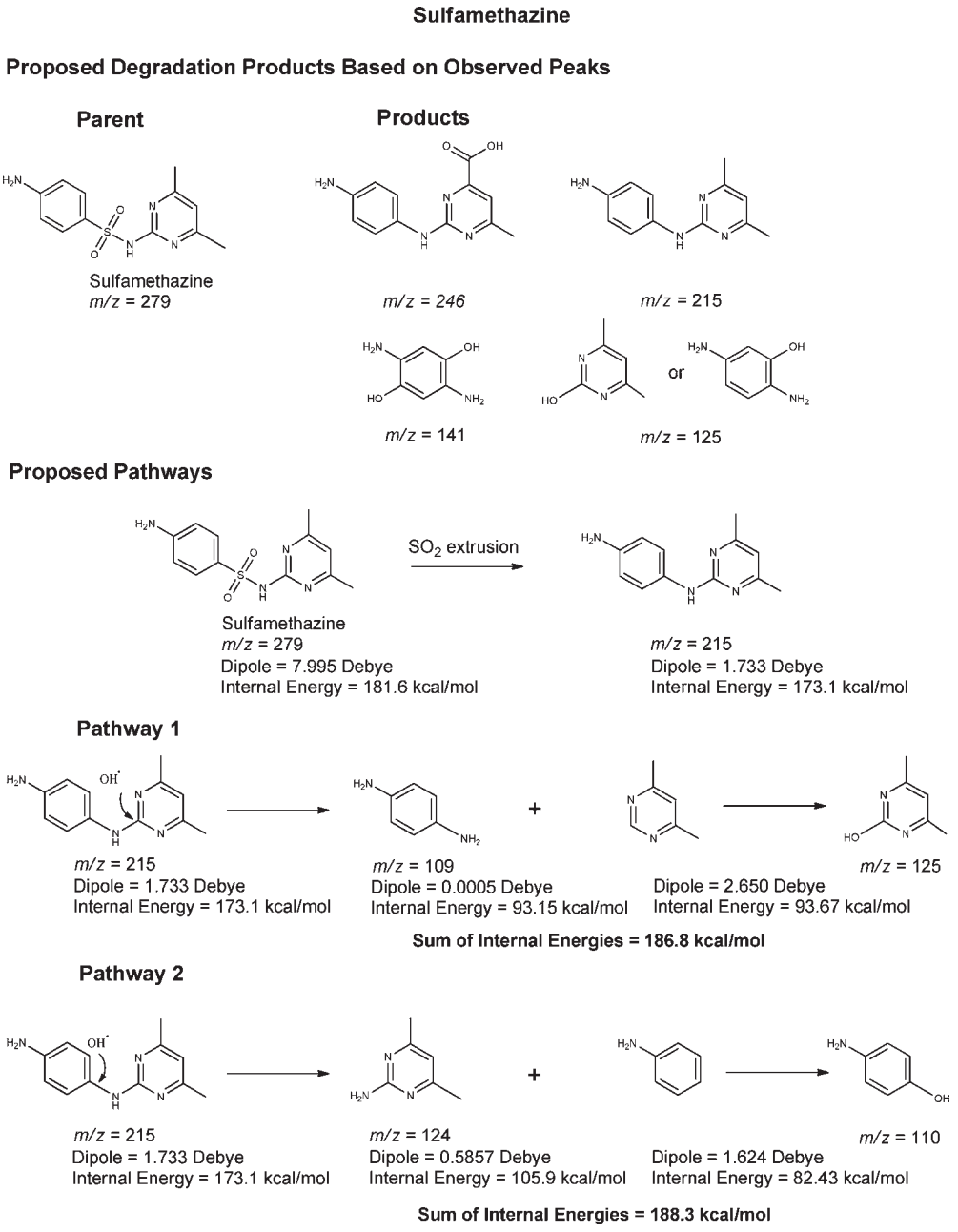

Figure 6. Proposed degradation pathways of sulfamethazine by hydroxyl radical attack in AFT.

Effect of Concentration on Degradation of Sulfamethazine. For AFT to be considered an effective and reasonable method for treating water contaminated with sulfonamides, it must be able to degrade sulfonamides at the various concentrations commonly found in the environment. Anodic Fenton treatment at the optimum H_2O_2/Fe^{2+} ratio and a fixed Fe^{2+} delivery rate of 38.85 μM min^{-1} was applied to sulfamethazine at initial concentrations between 18 and 250 μM, a range that is within that of accurate concentration analysis by HPLC and encompasses the concentration (143.7 μM) detected in European manure lagoons (*1*). Because the time to complete degradation decreased with lower initial concentrations (**Figure 4**), it can be inferred that AFT would rapidly degrade sulfamethazine at initial concentrations below the range tested, such as those found in groundwater in Europe and in U.S. rivers (*1*).

Effect of pH on Degradation of Sulfadiazine. AFT must also be able to rapidly degrade sulfonamides in water under a variety of pH values found in the environment. Sulfadiazine was chosen as the model compound to study the effects of initial pH on degradation, as it requires acidified water to dissolve, resulting in a stock solution pH of 2.3. In the Fenton reaction, the pH of the anodic half-cell drops to 2−3, which is advantageous to degradation because the acidity prevents the formation of iron precipitates that would interfere with the reaction (*29*). The modified AFT model fit the degradation of sulfadiazine with different initial pH values with regression coefficients of > 0.99 (**Figure 5**). The time at which sulfadiazine completely degraded was ~8 min for solutions with pH 3.1 and 7 and ~6 min for solutions with pH 4.3−6.3. The sulfadiazine solution with an initial pH of 5.1 completely degraded in the least amount of time, followed by the solutions with pH 4.3 and 6.3. Therefore, the optimum initial pH for the degradation of sulfadiazine by AFT is pH ~5.1. Overall, it was found that sulfadiazine degraded within 8 min in a solution at a near-neutral pH and that AFT can rapidly degrade sulfadiazine at a range of pH values typically found in aquatic environments (**Figure 5**).

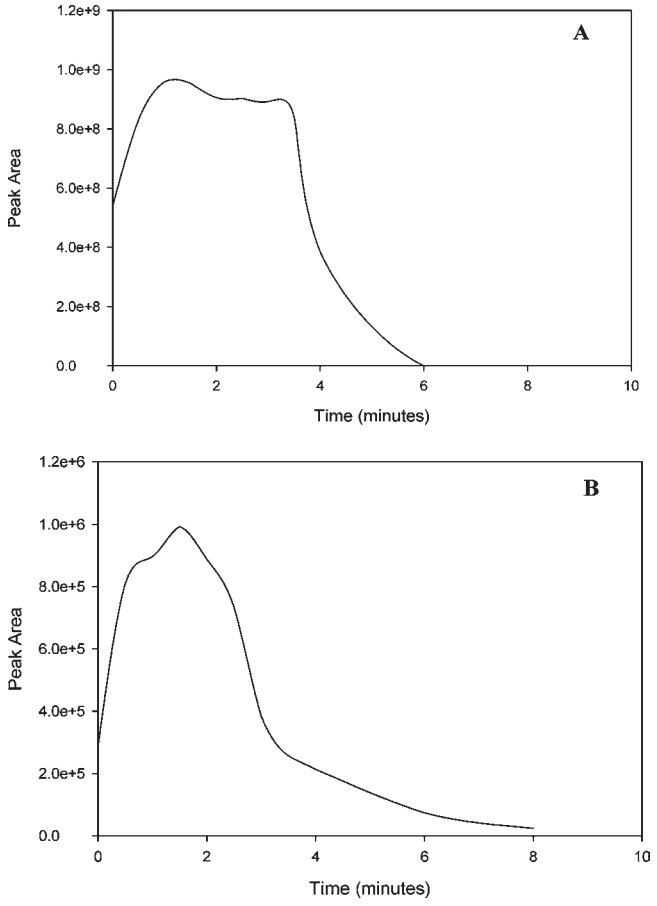

Figure 7. (**A**) Peak area of the sulfamethazine degradation product with *m*/*z* 215 throughout AFT. (**B**) Peak area of the sulfadiazine degradation product with *m*/*z* 187 throughout AFT.

Initial Degradation Products and Proposed Degradation Pathways of Sulfamethazine and Sulfadiazine. *Sulfamethazine*. A degradation pathway for sulfamethazine can be proposed on the basis of mass spectra and ab initio analysis of the byproducts of AFT (**Figure 6**). A molecular ion peak at *m*/*z* 279 was observed in the MS spectrum during the first 8 min of the AFT, which can be attributed to the parent compound, sulfamethazine; it decreases in peak area as the reaction proceeds (data not shown). A molecular ion peak at *m*/*z* 215 was observed in the spectrum from 0 to 4 min of the AFT, which appears to be the product of SO_2 extrusion, a phenomenon frequently exhibited by sulfonamides (*30*, *31*). The area of the *m*/*z* 215 peak increases from the beginning of the treatment to 2 min and decreases rapidly after 4 min (**Figure 7A**). This indicates that SO_2 extrusion is continuously occurring throughout the first 2 min of the AFT. A molecular ion with *m*/*z* 125 was observed during the first 6 min of the AFT; a molecular ion with *m*/*z* 141 was observed between 4 and 8 min; and a molecular ion with *m*/*z* 246 was observed between 2 and 4 min. These three molecular ions do not have measurable peak areas, indicating that, although they are probable degradation products, they are readily consumed by hydroxyl radicals and therefore do not accumulate in the system. Two likely pathways of degradation of the extrusion product with *m*/*z* 215 were analyzed using ab initio analysis. The first involves a hydroxyl radical attack at the carbon−nitrogen bond of the dinitrogen-substituted ring, which produces two products with *m*/*z* 109 and *m*/*z* 125 (**Figure 6**). The observed peak at *m*/*z* 125 could be either the product with *m*/*z* 109, to which a hydroxy group has been added, or the product with *m*/*z* 125. The second pathway involves a hydroxyl radical attack at the carbon−nitrogen bond of the benzene ring, which produces two products with *m*/*z* 124 and *m*/*z* 110 (**Figure 6**). The difference in the sum of the internal energies indicates the difference in the C−N bond dissociation energies and the tendency for the bond to break. The sum of the internal energies of the two products in the first pathway in their radical forms is 1.5 kcal/mol lower than that of the second pathway, a value high enough to suggest that the pathways may not occur simultaneously. This difference in internal energy, coupled with evidence in the mass spectrum that hydroxyl radicals substitute hydroxy groups for hydrogens on products of the first pathway (*m*/*z* 141, *m*/*z* 125), indicates that the first pathway is likely to be favored. The hydroxyl radical-induced degradation of diclofenac, a compound similar in structure to the extrusion product with *m*/*z* 215, involves the same two pathways postulated above (*32*). This indicates that cleavage at either side of the central nitrogen and the addition of a hydroxyl group to one of the resulting products is a plausible step in the degradation of *m*/*z* 215. The observation of a molecular ion with *m*/*z* 246 suggests that another pathway occurs concurrently, in which hydroxy groups are added to a methyl group of the nitrogen-substituted ring of the SO_2 extrusion product with *m*/*z* 215, forming a carbonyl group. Further ab initio analysis (data not shown) indicated that the most energetically favorable conformation for two hydroxyl radicals to add to the structure is as a carboxyl group on one of the methyl groups of the nitrogen-substituted ring.

Sulfadiazine. A degradation pathway for sulfadiazine can also be proposed on the basis of mass spectra and ab initio analysis (**Figure 8**). A molecular ion peak, assumed to be the parent compound sulfadiazine, was observed at *m*/*z* 251 during the first 8 min of AFT; it decreases in peak area as the reaction proceeds (data not shown). A molecular ion peak at *m*/*z* 187 was observed during the first 8 min and is assumed to be the product of SO_2 extrusion (*30*, *31*). The peak area of *m*/*z* 187 follows a similar trend as that of *m*/*z* 215 in sulfamethazine (**Figure 7B**). A molecular ion with *m*/*z* 125 was observed between 0 and 8 min, and a molecular ion with *m*/*z* 219 was observed between 0 and 1.5 min. As with the products of sulfamethazine, these two probable degradation products do not have quantifiable peak areas, indicating that they are consumed by hydroxyl radicals in the system. As with sulfamethazine, there are two possible breakdown pathways from the SO_2 extrusion product. In the first pathway, a hydroxyl radical attacks the carbon−nitrogen bond of the dinitrogen-substituted ring of the SO_2 extrusion product, forming two products with *m*/*z* 109 and *m*/*z* 97. In the second pathway, a hydroxyl radical attacks the carbon−nitrogen bond of the benzene ring of the SO_2 extrusion product, forming a product with *m*/*z* 110 and a product with *m*/*z* 96. The difference in the sum of the internal energies of the two products in their radical forms between both pathways is 0.227 kcal/mol. This value is not high enough to be significant, so it is likely that both pathways occur. As with the extrusion product of sulfamethazine, the extrusion product of sulfadiazine with *m*/*z* 187 is similar in structure to diclofenac (*32*). Because diclofenac degrades according to the two mechanisms described above, the proposed degradation pathways for the molecular ion with *m*/*z* 187 are plausible. The observation of the molecular ion with *m*/*z* 219 suggests that another pathway occurs concurrently, in which hydroxy groups are added directly to the nitrogen-substituted ring of the SO_2 extrusion product with *m*/*z* 187.

Removal of Bacteriostatic Activity by AFT: Toxicity Test with *L. gibba*. The success of AFT depends not only on the complete removal of sulfamethazine from the system but also on the removal of any degradation products with bacteriostatic properties.

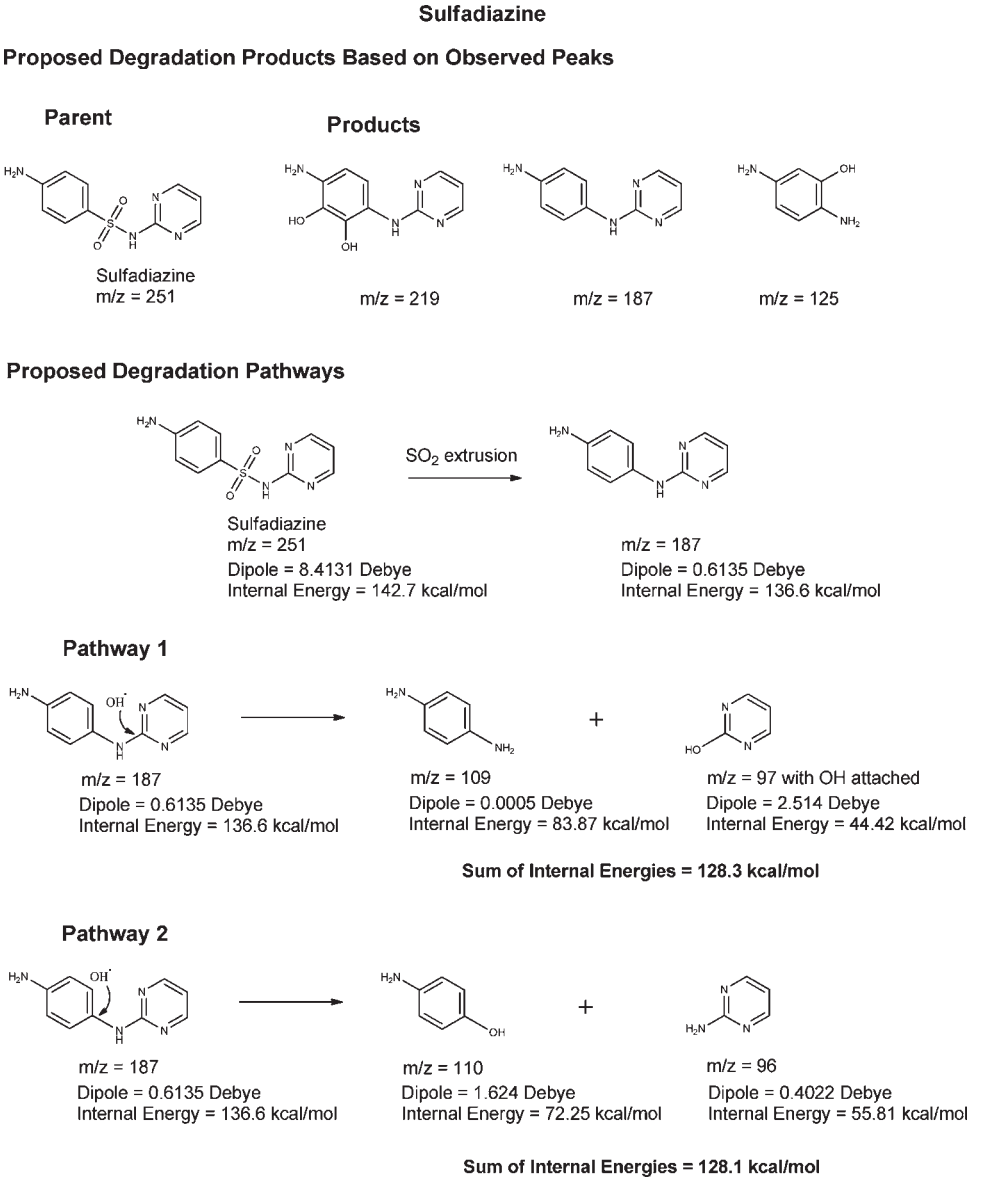

Figure 8. Proposed degradation pathways of sulfadiazine by hydroxyl radical attack in AFT.

Table 1. *p* Values Associated with Dunnett's *t* Test, Which Determines Whether the Wet Weights of *L. gibba* in Experimental Units (AFT with and without Sulfonamide) Are Significantly ($p < 0.05$) Lower than Control

treatment	*p* value	treatment	*p* value
AFT run without sulfamethazine		**AFT run without sulfadiazine**	
0 min	0.997	0 min	1.000
2 min	0.902	2 min	1.000
4 min	0.985	4 min	1.000
6 min	0.657	6 min	1.000
15 min	0.981	15 min	1.000
AFT run with sulfamethazine		**AFT run with sulfadiazine**	
0 min	0.000	0 min	0.000
2 min	0.006	2 min	0.000
4 min	0.012	4 min	0.000
6 min	0.270	6 min	0.000
15 min	0.936	15 min	0.998

As mentioned previously, SO_2 resembles the carbonyl group in PABA (**Figure 1**) and confers reactivity to the aromatic amine (*9*). In the proposed degradation schemes, SO_2 is removed from the ring and hydroxy groups are added. The replacement of the active sulfonyl group with a primary amine group or hydroxy group may decrease the activity of the aromatic primary amine and the overall bacteriostatic activity of the compound (*9*). The addition of hydroxy groups and/or carbonyl groups and their corresponding electron orbitals, as well as the removal of the large electron orbital of the sulfonyl group may also decrease the sulfonamide's bacteriostatic activity, because the shape of the degradation product would likely interfere with its ability to bind to bacterial enzymes involved in folate synthesis (*9*).

To test whether AFT removed the bacteriostatic properties of sulfonamide during degradation, *L. gibba* was exposed to AFT effluent from various time points in the reaction for 7 days. The goals were to determine whether exposure to the sulfonamide-containing AFT effluent at the beginning of the reaction (0 min) would result in significantly lower wet weights than the control and whether exposure to effluent near the end of the reaction (15 min) would result in wet weights that are not significantly different from the control.

The validity of the experiment was confirmed by the observation of at least a 7-fold increase in total frond number in all controls at the end of the experimental period (*27*). Experimental

units exposed to sulfonamide-free AFT effluent did not have significantly lower wet weights than controls at any time during AFT (**Table 1**). Therefore, any toxicity observed in experimental units exposed to sulfonamide-containing AFT effluent is due only to the presence of the sulfonamide.

Experimental units exposed to sulfamethazine-containing AFT effluent from 0, 2, and 4 min had significantly lower wet weights than the control, whereas those exposed to effluent from 6 and 15 min did not (**Table 1**). These observations are in accordance with previously established wet weight EC_{50} values for sulfamethazine in *L. gibba*. The initial concentration of sulfamethazine in the anodic half-cell was 1122 μg/L, and the published EC_{50} is 1277 μg/L. The concentration in the anodic half-cell at 15 min was 10 μg/L, and the published EC_{10} is 381 μg/L (*25*). Therefore, although AFT did not completely degrade the initial concentration of sulfamethazine within 15 min, the concentration of active sulfamethazine after 15 min of degradation was too low to elicit a significant toxic response.

Similarly, experimental units exposed to sulfadiazine-containing AFT effluent from 0, 2, 4, and 6 min had significantly lower wet weights than the control, whereas those exposed to effluent at 15 min did not. The initial concentration of sulfadiazine in the anodic half-cell was 1107 μg/L, and the concentration at 15 min was 118 μg/L. Although EC_{50} values have not been established for sulfadiazine, it was found in a separate experiment that exposure to 579 μg/L resulted in significantly ($p < 0.05$) lower wet weights than the control, whereas exposure to 46 μg/L did not. The results of these toxicity tests support the hypothesis that AFT removes the bacteriostatic properties of sulfamethazine and sulfadiazine during degradation.

Conclusions. AFT fully degraded 100 μM sulfamethazine in aqueous solution at a Fe^{2+} delivery rate under optimal conditions within 6 min and is expected to degrade sulfamethazine at concentrations found in contaminated rivers and groundwater within 10 min. AFT completely degraded 100 μM sulfadiazine under optimal conditions at a range of pH values likely to be found aquatic environments within 6−8 min of treatment.

During AFT, the sulfonyl group was removed and hydroxy groups were added to the extrusion product, which was fragmented and modified, producing degradation products with potentially reduced bacteriostatic capabilities. Toxicity tests with *L. gibba* indicate that AFT removes the bacteriostatic properties of sulfamethazine and sulfadiazine during degradation.

ABBREVIATIONS USED

AFT, anodic Fenton treatment; HPLC, high-performance liquid chromatography; LC-MS; liquid chromatography−mass spectrometry; GAMESS, General Atomic and Molecular Electronic Structure System.

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